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THE TOXICOLOGY OF OZONE AND SULFURIC ACID AEROSOL DURING THEIR COMBINED EFFECT

[K toksikologii ozona i aerozolya sernoy kisloty pri kombinirovannom deystvii]

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THE TOXICOLOGY OF OZONE AND SULFURIC ACID AEROSOL DURING THEIR COMBINED EFFECT

A.I. Nevskaya and T.A. Kochetkova*

The basic harmful factors in hydrogen peroxide manufacturing shops are the emissions of ozone and sulfuric acid aerosol, which enter the air of industrial areas in case of seal break in apparatus and communications from the raw and intermediate solutions throughout the entire course of the technological process.

In addition to diseases of the respiratory organs (pneumonia, bronchites, bronciolites, etc.), diseases of the liver, weight loss, and the development of pneumosclerosis have been identified in a number of workers involved in producing hydrogen peroxide over a 2 - 3 year period.

The absence of hydrogen peroxide vapor in the air of working areas gives basis to assume that the diseases noted in the workers can be attributed to the combined effect of ozone and sulfuric acid aerosol, which were constantly detected in the air of the working areas.

The concentrations of ozone in working areas and the production of hydrogen peroxide with open electrolyzers were within limits of 0.00016 - 1.0018 mg/l, and 0.00003 - 0.0009 mg/l with enclosed electrolyzers (A.I. Nevskaya and D.D. Diterikhs).

Data of the literature indicate the high toxicity of each of the named products individually (N.V. Lazarev, S.M. Gorodinskiy, K.A. Bushtuyeva, Stoking-

*(Moscow). The Institute of Occupational Hygiene and Occupational Diseases of the Academy of Medical Sciences of the USSR.

der, Amdur, Schulz and Drinker, Goldman, and others [Stokingder, Amdur, Shulz and Drinker, Goldman and others]).

The effect of a mixture of ozone and sulfuric aerosol on the organisms of animals and man has not been studied up to now. There are experimental works devoted to investigating the toxicity of ozone in a mixture with oxides of nitrogen, ozone with carbon dioxide, sulfuric acid aerosol with sulfur dioxide, etc.

Thorp showed that the toxicity of ozone sharply increases with the presence of the oxides of nitrogen in the air. According to the data of Mittler, Herdrick and Phillips, carbon dioxide enhances the toxicity of ozone. Amdur showed that respiratory disorders ensue in guinea pigs with the combined effect of sulfur dioxide (0.18 mg/l) and sulfuric acid (0.008 mg/l), and the animals are stunted in growth more rapidly than during the exposure of the animals to each product individually in the same concentrations.

As is apparent from the cited studies, the harmful effect of ozone or sulfuric acid aerosol on the organism of animals is enhanced in the presence of other products. The purpose of our investigations was to study the combined effect of ozone and sulfuric acid aerosol on animals.

The effect of the indicated mixture was studied in short-term and long-term experiments. Acute poisonings of animals were carried out on albino mice (460 animals) and albino rats (190 animals). A total of 50 experiments were carried out in which the toxicity of the studied mixture and its component products was investigated during single two-hour exposure.

We use the electrolytic method to obtain ozone. During passage of oxygen into the ozonator, electrical discharges occurred on the electrodes with the formation of ozone.

Sulfuric acid aerosol was obtained by means of supplying dry sulfur dioxide to the chamber, and as the result of the reaction of the sulfur dioxide with

moist air in the chamber, condensation aerosol with high dispersion was obtained. The actual concentrations in the chamber were investigated chemically by means of the titrometric method to determine ozone, and the nephelometric method to determine sulfuric acid aerosol.

On the basis of the data of the short-term experiments, absolutely lethal concentrations of ozone and sulfuric acid aerosol were obtained for mice and rats. The mortality data of albino mice and rats with the studied poisons were integrated according to Berens and the mean lethal concentrations were obtained (LC_{50}) (Figure 1). The results of these experiments are given in the table.

As is apparent from the table, mice are approximately 1.5 times less sensitive to ozone than rats, and, on the other hand, are 1.5 times more sensitive to sulfuric acid.

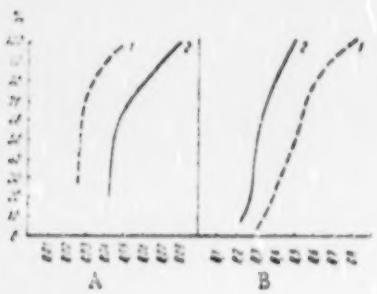

Figure 1. The mortality curve (mortality rate) of albino rats and mice with increasing concentrations of ozone and sulfuric acid aerosol. Along the axis of the abscissa - concentrations in milligrams per 1 (A- Ozone; B- Sulfuric acid aerosol); along the axis of the ordinate - percentage of animals that died.
1 - Rats; 2 - Mice.

High concentrations of sulfuric acid aerosol (0.2 - 0.5 mg/1 for mice and 0.4 - 0.8 mg/1 for rats) cause the following symptons in the experimental animals: a period of excitation that lasted from 5 to 7 minutes replaced by deep sleep, weeping, and a sanious secretion from the nose, gradually increasing dyspnea, and motor coordination disorders. The condition of the animals was serious. Occasionally 1 - 2 animals died during exposure. After exposure, the animals were listless, immobile, had poor appetite, and their coats remained disheveled for a day. Well-being gradually improved in the surviving animals; 2 - 3 days later they became active and ate better, but the coat remained disheveled for the next few days.

High concentrations of ozone (0.03 - 0.08 mg/l for mice and 0.02 - 0.05 mg/l for rats) caused the same symptoms in the experimental animals that were observed during the effect of sulfuric acid aerosol, but were more strongly pronounced. The period of excitation lasted from 3 to 5 minutes; a large number of deaths during exposure were also recorded in the first days of poisoning. The death of the remaining animals (50-100%) was noted over the course of the first three days. At the autopsy of the animals, sharp plethora of the internal organs was noted.

ABSOLUTE AND MEAN LETHAL CONCENTRATION OF OZONE AND SULFURIC ACID AEROSOL FOR MICE AND RATS

Animals	Absolute lethal concentrations (in mg/l)		Mean lethal (LC_{50}) concentrations (in mg/l)	
	Ozone	Sulfuric acid aerosol	Ozone	Sulfuric acid aerosol
Mice	{ 0,060 0,080	{ 0,45 0,5[illegible]	{ 0,041 0,046	{ 0,2[illegible] 0,32
Rats	{ 0,045 0,06[illegible]	{ 0,77 0,8[illegible]	{ 0,023 0,02[illegible]	{ 0,45 0,54

The histological investigation, conducted with the consultation of Professor P.P. Dvizhkov, showed the following.

The first few days after exposure to sulfuric acid aerosol, disorders of the blood and lymph circulation are observed in the form of sharp plethora of the internal organs and individual small hemorrhages in the lungs and brain (in certain animals - in the spleen and kidneys).

A local stimulating effect in the lungs was expressed in swelling of the bronchial mucosa, its partial desquamation, and expansion of the bronchial lumina. Focal bronchial pneumonia developed in most animals. In the animals killed 6 and 12 months later, symptoms of chronic catarrhal tracheitis, bronchitis, slight peribronchial and perivascular sclerosis were observed; there was a diffuse chronic, interstitial, frequently pronounced process (an increase in the number of cells of a histiocyte and lymphoid character with a mixture

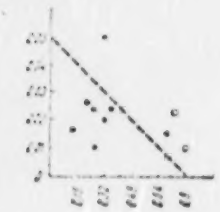

Figure 2. The combined effect of ozone and sulfuric acid aerosol on the rats (the concentration of each gas, producing death of the animals in 100% of the cases, are accepted as the effective ones). Along the axis of the abscissa - concentrations of sulfuric acid aerosol in milligrams per 1 ml; along the axis of the ordinate - concentrations of ozone in milligrams per 1 ml; circles - positive experiments; squares - negative experiments.

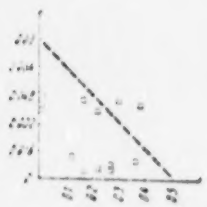

Figure 3. The combined effect of ozone and sulfuric acid aerosol on mice. Symbols are the same as in Figure 2.

of polynuclears and the appearance of collagen fibers). Small focal emphysema was pronounced.

All these symptoms were more sharply pronounced in the experimental animals that had died as the result of acute exposure to ozone and in those killed after 6 and 12 months. Moreover, homogenization of the epithelium of the bronchial mucosa is observed, in places with total absence of the latter in the early periods of exposure; and in the latter period there is often sharply pronounced sclerosis around the central veins of the liver.

After establishing the lethal concentrations for each component individually, experiments were conducted with a mixture of ozone and sulfuric acid aerosol (Figures 2 and 3). The diagrams that show the data we obtained are plotted according to Lowe, who suggested the graphic method of depicting the combined effect of poisonings. The figures are an "isodynamic field," according to Lowe, and are plotted such that the different proportions of effective (accepted as 100%) concentrations of the studied gases (i.e., proportions of the concentrations which produce the death of animals in 100% of the cases) are laid-off on the axes of the ordinates. The positive experiments (death) are depicted by circles and the negative ones - by squares. The line that joins the points of identical effect, according to Lowe, is called an "isobola." When the studied effect is observed at such minimum concentrations of each of the mixture components that, being expressed in percentages of the effective concentration of each gas individually, they total 100% when the points that depict the positive experiments are plotted along the diagonal (the dotted

line in the figures, representing the simplest case of a theoretical isovol of simple addition, according to Lowe). When the positive effect is obtained with a concentration of mixture in the total exceeds 100%, the positive points are located above the diagonal (antagonism of effect), and in the case of synergism - below the diagonal.

Figure 2 shows the results of the experiments with the combined effect of ozone and sulfuric acid aerosol performed on rats. In this case the points that depict the positive experiments, i.e., when the death of the animals ensued during exposure or over the course of three days after exposure, are arranged both above the diagonal and below the diagonal. The different combinations of concentrations of the separate components, in the sum of components 50, 80, 100 and 140%, produce a positive effect (100% mortality rate of the animals). These experiments showed that the given mixture not only has a cumulative effect but also has a synergistic one (enhancement of effect) in rats. As is apparent in Figure 3, different combinations of the separate gases comprising a total of 40, 55, 90, and 130% produced a negative effect. All experiments were negative, even in those cases when the concentrations of both gases in toto exceeded 100%. Hence, an antagonism of effect was observed during the combined effect of gases on mice.

The species-specific difference was confirmed by experiments in which mice and rats were simultaneously exposed to the effect of a mixture of ozone in a concentration of 0.023 mg/l and sulfuric acid aerosol in a concentration of 0.5 mg/l. Death of the rats was also observed in 70 - 90% of the cases, under these conditions, while at the same time there was no mortality of the mice. Based on the preceding data, in these experiments the concentration of ozone of 0.023 mg/l was the LC_{50} for mice and approximately half LC_{50} for rats; the concentration of sulfuric acid aerosol of 0.5 mg/l was LC_{50} for rats and approximately half the LC_{50} for mice.

During the investigation of toxicity of the mixture, the following was found: in the experimental animals that were exposed to the simultaneous effect of ozone and sulfuric acid aerosol (in concentrations near LC_{50} a

shorter one was observed - up to 1 minute), the period of excitation was replaced by deep sleep. The condition of the animals was still more serious than under the effect of each of the individual components of the mixture. Dyspnea, which appeared at the beginning of the experiment, noticeably and rapidly strengthened; disorders of motor coordination rapidly increased; the animals often lay on their sides, inhaling air with great effort. After the end of 2 hours of poisoning, the animals were in a more serious state (deep and continuous sleep), than in the preceding experiments; the animals were seemingly in a state of narcosis. During subsequent observation, a sharp difference was found in the behavior of the mice and rats. Several hours after exposure, the general condition of the mice improved and the next day they did not outwardly differ from the control, while the condition of the rats noticeably deteriorated. Over the first 3 days, 50 - 100% of the experimental rats died, while mortality for mice over the same period did not exceed 27 - 33%.

At autopsy of the experimental animals poisoned with a mixture of these products, plethora of the internal organs, foci of pneumonia and pulmonary edema were noted. The histological investigation demonstrated sharply pronounced symptoms of disorders of the blood and lymph circulation (severe plethora and edema of the internal organs, hemorrhages into the substantia and pia matter of the brain, in the lungs, kidneys and spleen) and suppurative-destructive process of the bronchi and lungs (Figure 4). In individual animals necrotic and necrobiotic changes were observed in the liver.

In animals that died after 3 and 6 months, a catarrhal-desquamative, sometimes suppurative, bronchitis is detected, with mildly pronounced peribronchial, perivascular and initial small focal interstitial sclerosis, which slowly progressed but did not reach a significant degree of pronouncement even after 12 months.

Hence, the results of the short-term experiments made it possible to establish a different species-specific sensitivity of animals with respect to ozone and sulfuric acid aerosol, as well as the mixture of these compounds.

Figure 4. The lung of an albino mouse killed on the first day after inhalation poisoning with a mixture of the substances. Necrobiotic and necrotic changes of the bronchial mucosa (magnification 8 X 40).

The rats were more sensitive than the mice both to the mixture and to ozone. Moreover, enhancement of the toxic effect of the mixture of ozone and sulfuric acid was found in rats in comparison with the effect of each of the named products individually.

It was our idea to verify the data obtained in short-term experiments on rats and which indicate that the mixture of ozone and sulfuric acid aerosol is more toxic than the sum of concentrations of the separate components by means of the long-term experiments.

When making the mixture of ozone and sulfuric acid aerosol, we used concentrations of these products slightly lower than the threshold ones (N.V. Lazarev, S.M. Gorodinskiy, K.A. Bushtuyeva, and others). Simultaneously with the chronic poisonings of animals with a mixture, chronic poisonings of animals with the separate components were carried out.

The chronic effect of ozone was studied with a mean concentration of 0.0008 mg/l (± 0.0003); sulfuric acid aerosol - 0.007 mg/l (± 0.003), and their mixtures - in a combination of the two indicated concentrations (the mean concentrations were calculated by the method of variation statistics).

In each of the three series of experiments and in the control group there were 10 rats weighing from 270 to 280 grams. The dynamic method of poisoning was employed. The experiments were carried out in chambers having a 100 liter volume.

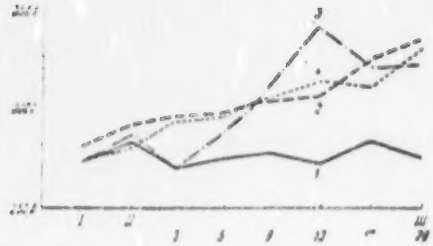

Figure 5. Curves of weight of the animals during chronic poisoning with ozone, sulfuric acid aerosol, and their mixture. Along the axis of the abscissa - time of exposure (I - before experiment; II- beginning of the experiment; III- end of the experiment); along the axis of the ordinate - weight of the animals in grams; 1- poisoning with mixture of the substances; 2- ozone poisoning; 3- poisoning with sulfuric acid aerosol; 4- control.

Poisonings were carried out for 6 days a week with a length of daily exposure of 5 hours. The total duration of exposure was 4 and 1/2 months (100 days).

The indicators of intoxication in all animals were viability, behavior of the animals, change in weight of the animals, composition of the peripheral blood, the functional condition of the central nervous system with respect to its capacity to summate stimuli, oxygen consumption, and pathomorphological changes of the internal organs.

Over the period of long-term exposure, the mortality rate of rats was approximately identical in all series of experiments: in the experiment with ozone, 2 animals died; in the experiment with sulfuric acid aerosol, 2 animals died; in the experiment with the mixture - 3 animals died, and in the control, 2 animals died. The immediate causes of death were unrelated to the effect of the studied products and 2 were due to intercurrent diseases. However, upon autopsy of all of the killed experimental animals, unlike the control, inflammatory changes were noted in the lungs and were somewhat more pronounced in the animals that had been exposed to the action of the mixture. The animals that survived did not differ outwardly from the control. During the period of long-term exposure to the studied substances, all of the experimental animals did not show outward noticeable deviations from the norm, but during the investigation a clear change in a number of indicators was established.

Changes in weight of the experimental rats in all 3 series of experiments are given in Figure 5. The weight of the animals exposed to the effect of the mixture of ozone and sulfuric acid aerosol significantly lagged behind

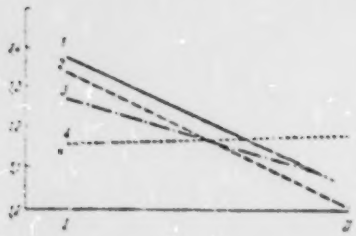

Figure 6. Change in the amount of consumed oxygen in rats during chronic poisoning with ozone, sulfuric acid aerosol and their mixture. Along the axis of the abscissa - exposure time (I- beginning of the experiment, II- end of the experiment); along the axis of the ordinate - amount of consumed oxygen in milliliters; 1- poisoning with a mixture of substances 2- ozone poisoning; 3- poisoning with sulfuric acid aerosol; 4- control.

both the weight of the control group and the weight of the experimental animals that were exposed to the action of the separate components; the weight of the last 2 groups differed little from that of the control animals.

During the first periods of intoxication, noticeably pronounced inhibition (particularly in the group of animals exposed to the action of the mixture) was noted in all of the experimental animals, which is indicated by the reduced excitability (the reflex reaction appears with a large number of stimuli). In the later periods of exposure, inhibition becomes less noticeable, and in the group of animals exposed to the action of the mixture an increase in excitability of the central nervous system is noted. In the same periods of observations, no noticeable deviations from the original level were noted in the control group of animals.

No significant changes were observed with respect to the blood.

In all of the groups of experimental animals, reduced oxygen consumption was noted toward the end of the poisoning period, and a more pronounced reduction was noted under the effect of ozone and the mixture of substances (by 25 and 22%, respectively). In the control group no reduction in oxygen consumption was noted over the course of the entire observation period (Figure 6).

Histological investigation: with chronic inhalation poisonings with a mixture of ozone and sulfuric acid aerosol, chronic catarrhal-desquamative, partly suppurative tracheitis, bronchitis; peribronchial, perivascular and interstitial sclerosis noted after 2 and 1/2 months and gradually increasing toward the fifth month develop; in the lungs - chronic diffuse interstitial,

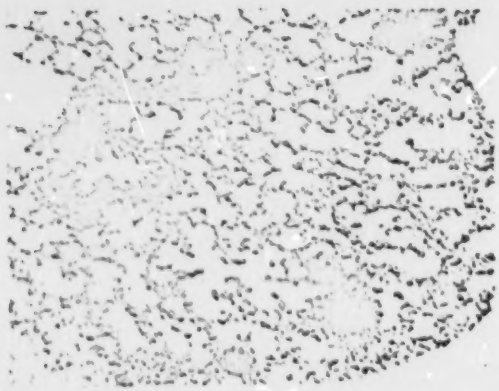

Figure 7. The lung of an albino rat killed 2 and 1/2 months after inhalation poisoning. Catarrhal-desquamative bronchitis, slight peribronchial and perivascular sclerosis. Hyperplasia of the lymphoid tissue. Widespread thickening of the septa. Slight small focal emphysema (magnification 8 X 10).

slightly pronounced inflammatory process (chronic interstitial pneumonia). Slight small focal emphysema (Figure 7).

During chronic inhalation poisonings with separate components of the mixture, the histologic investigation shows changes similar to those described above but less sharply pronounced with ozone and still less under the effect of sulfuric acid aerosol. Thus, for example, under the effect of sulfuric acid aerosol the development of connective tissue in the lungs is noted only after 5 months, under the effect of ozone - after 3 - 3 and 1/2 months, and under the effect of the mixture - after 2 and 1/2 months.

Hence, our experiments showed that ozone and sulfuric acid aerosol, even in concentrations near the threshold effective ones, caused changes in animals not only in weight, amount of consumed oxygen, the functional condition of the summative capacity of the central nervous systems, but also pathomorphological changes of the internal organs.

All of these indicators of intoxication were more pronounced in the group of experimental animals exposed to the combined effect of ozone and sulfuric acid aerosol.

The results of the experimental investigations are in agreement with the data of clinical observations of the state of health of workers involved in the production of hydrogen peroxide, where ozone and sulfuric acid aerosol are constantly present in the air of working areas. Irritation of the respiratory passages, bronchites, pneumonia, etc. are significantly more pronounced in workers under the effect of this mixture than with the same

concentrations of each component individually.

CONCLUSIONS

1. During the short-term effect of a mixture of ozone and sulfuric acid aerosol in different combinations, enhancement of toxic effects was found in rats in comparison with the effect of each of the individual components.

2. Enhancement of the toxic effect was found during long-term exposure to a mixture of ozone and sulfuric acid aerosol in concentrations near the threshold concentration.

3. A different species-specific sensitivity was shown in mice and rats with respect to the combined effect of ozone and sulfuric acid aerosol and to each component individually.

4. The above enables one to recommend lowering the magnitude of maximum permissible concentrations of ozone and sulfuric acid aerosol by 2 with their simultaneous presence in the air of production areas.

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ON THE TOXICOLOGY OF OZONE AND AEROSOL OF SULPHURIC ACID IN THEIR COMBINED ACTION

[illegible] Nefedova, Z. A. Kucheriavaia (Moscow)

Summary

[illegible] action of a mixture comprising different proportions of [illegible] and a strengthening of the toxic effect could be revealed [illegible] by each one of the above components, taken separately.

[illegible] the toxic effect was observed also during chronic [illegible] ozone and aerosol of sulphuric acid in concentrations approaching the threshold ones.

Varying species sensitivity was established to mice and rats to the combined action of ozone and aerosol of sulphuric acid and to the effect of each one of these elements taken separately.

This makes it advisable to suggest cutting in half the values of maximal permissible concentrations of ozone and aerosol of sulphuric acid, when they are present together in the atmosphere of industrial premises.